CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER
Advancing Protein Science for Personalized Medicine

eProtein

Letter from the Director

Dear Colleagues,

In June of this year, members of the Clinical Proteomic Technologies for Cancer (CPTC) community provided a program update to NCI's Board of Scientific Advisors (BSA), which was very well received. The BSA, consisting of 35 members from a number of disciplines in science and medicine, advises NCI's senior leadership on a wide variety of matters concerning scientific program policy and the progress and future direction of extramural research programs.

The presentations were especially focused on the more robust and efficient protein biomarker development pipeline that has been developed by this initiative. Simply said, CPTC is restructuring this pipeline to include a verification, or pre-validation step, which serves as a bridge between biomarker discovery and clinical validation. Verification may provide a very reliable GO/NO GO decision point and potentially save the medical diagnostic industry millions of dollars and many years of development because only the strongest candidates will move into clinical validation—and with much greater confidence.

In just a few short years, CPTC investigators have made significant advances in the field that will affect the way every investigator does protein biomarker discovery research. To learn about this endeavor, and other tremendous advances being made by this initiative, including community resources and data release policies, I encourage you to attend our upcoming annual meeting this October. I look forward to seeing you there! ■

Under-Represented Students: Training the Next Generation of Cancer Research Scientists

The National Institutes of Health Emerging Technologies Continuing Umbrella of Research Experiences (ET CURE) pilot program addresses the need for a diverse cancer research community in the 21st century to reflect the nation's ethnic heterogeneity. In support of ET CURE, NCI's Center to Reduce Cancer Health Disparities (CRCHD)

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An Advocate's Perspective

A Response to "The Promise and the Reality of Proteomics" Webinar

Elda Railey
Co-Founder
Research Advocacy Network

For years, we have been promised more personalized medicine and targeted therapies for cancer, and today we have learned more of the real promise for cancer detection and treatment through the study of proteomics. The issue of cost savings through early detection methods is very important as the strain on our

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A Clinical Proteomic Technologies for Cancer initiative publication that builds connections throughout the proteomics community

In This Issue

Under-Represented Students: Training the Next Generation of Cancer Research Scientists

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has provided principal investigators with the opportunity to plan and implement a research training program in emerging technologies through research supplements for high school and undergraduate students from underserved populations.

The goals of the ET CURE initiative are to:

1. Create a pipeline of underserved students and investigators in the fields of emerging and advanced technologies;
2. Increase the number of scientists from underserved populations with training in the elective disciplines of focus, such as nanotechnology, clinical proteomics, bioinformatics, biophotonics, and cancer health disparities;
3. Enhance the application of emerging technologies to cancer research through increased training and educational opportunities; and
4. Foster academic, scientific, and multi-disciplinary research excellence to culminate the emergence of a mature investigator capable of securing competitive advanced research funding.

There are a number of domestic institutions involved in the ET CURE initiative. In this pilot program, LeeAnn Bailey, Ph.D., Program Director of CRCHD, will determine how each institution recruits students, the types of applicants they receive, the types of programs that have been set up, and what proves successful. The pilot will then be followed by a larger scale program.

Amanda Paulovich, M.D., Ph.D., of the Fred Hutchinson Cancer Research Center (FHCRC) and a member of the Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network, is an active participant in the ET CURE program. "My lab is involved in clinical proteomics, and our goal is to give budding students exposure to the field," explains Paulovich.

> *"I was given tremendous research opportunities as an undergraduate student at Carnegie Mellon University, and I want to give that back."*

The original plan was to have a single undergraduate student from an underserved or minority population join the Paulovich lab to gain exposure to clinical proteomic technologies. This plan changed, however, when three applications in particular stood out.

"One is a biology major, one a chemistry/biology double major, and one a computer science major. This stuck out to me because in real projects in the lab that are funded on grants, we tend to have a three person team: a biochemist or biologist, a chemist, and a bioinformaticist," outlines Paulovich. "We thought, wouldn't it be fun to put these three together as a team? So that's what we did."

The first three students included Brianna Byers and Tim Nguyen, both from the University of Washington, and Christina Tieu from Pacific Lutheran University. Christina recently left FHCRC to continue a successful career in biomedicine through the M.D./Ph.D. program at the Mayo Clinic. A local high school student, Tao Large, from The Northwest School, has since joined the lab as her replacement.

The curriculum for these students includes four separate activities that are geared towards preparing them for a successful career in biomedical research.

(From left to right) Tao Large, Tim Nguyen, and Brianna Byers

First, the students are working collaboratively on a project using state-of-the-art proteomic approaches to discover cellular responses to DNA damage. This project has been designed to cover topics spanning basic biology through advanced mass spectrometry and data analysis. Every member of the Paulovich lab is actively engaged in assisting the students in these experiments. "When they came into the lab, the students received a list of topics they would touch on during their experiments as well as a roster identifying the appropriate contact person in the lab that the students could talk to about each of the relevant topics," says Paulovich. "This prevented just one person from getting overwhelmed with responsibility while also ensuring that the students develop relationships with all of the lab members. Hence, the whole lab is taking part in the effort, helping the students navigate the experiments from beginning to end."

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Second, the students are also developing their presentation skills. Paulovich has provided the students with a list of topics on which they will prepare presentations and deliver them to the entire lab group, which currently includes 25 members. "The students recently gave their first round of presentations, and they exceeded my expectations! It is very invigorating to have their energy and enthusiasm around the lab," says Paulovich.

Third, in order to give the students a real taste of what it means to be in biomedical research, they are gaining critical experience in the NIH grant application process. Following a crash course in this process (given by Paulovich), the students are completing a mock grant application that assumes they have to convince a panel of reviewers to fund their clinical proteomics project. "This exercise is useful not only for exposing them to the grant process but also for forcing them to think critically about their experiments and to learn relevant background information related to their project," notes Paulovich.

And finally, the students are taking part in weekly faculty seminar series and writing workshops that have been set up for all summer interns at FHCRC. This provides them with the opportunity to network with faculty members and other interns.

"The critical thing is to get them excited about the field," explains Paulovich. "I was given tremendous research opportunities as an undergraduate student at Carnegie Mellon University, and I want to give that back." ■

An Advocate's Perspective

A Response to "The Promise and the Reality of Proteomics" Webinar

(continued from cover)

Research Advocacy Network

healthcare system is widely felt. However, the cost savings of early detection methods are negated when the results are not reliable, or the costs of the tests outweigh their validity.

Part of the "omics" promise is the development of better candidates for drug therapies and early cancer diagnostics for improved cure rates and reduced costs of treatment, both in human suffering and in dollars. This promise is especially enticing in harder to diagnose cancers such as squamous cell head and neck cancer.

I must admit that I was still confused about the difference between genetics/genomics and proteomics. After studying the materials from NCI, I have a better understanding that genes give a glimpse of what MAY occur, and proteomics can help understand what is happening in REAL TIME. The reality for a patient is that it is not important what type of technology or "omics" science results in the best detection methods and personalized treatment choices, but it is very important to patients that the results returned by these technologies are accurate and reliable.

When donating biospecimens for research, it matters that the "piece of me," whether it is blood, serum, or another biospecimen, is used to gain the maximum amount of information and contribute to the knowledge base to fight cancer. We also want to be assured that our privacy is protected.

Even though I have been in cancer advocacy for many years, it was not easy to make proteomics a concept relevant to our everyday lives. Yet this area is where much of the research investment dollars and the state of science are headed. It was published recently that colon cancer patients who knew about targeted therapies were more likely to receive those treatments. We will probably find that patients who know about these important early detection methods are more likely to utilize them.

For advocates to be helpful we do need to understand what the proteomics pipeline really contains and what the outcomes of the work have been in the past. We need to understand what the barriers and promoters of the knowledge are along the way, and how researchers across disciplines are sharing these technologies and knowledge so that these methods can be integrated into clinical practice.

> *"The reality for a patient is that it is not important what type of technology or "omics" science results in the best detection methods and personalized treatment choices, but it is very important to patients that the results returned by these technologies are accurate and reliable."*

We hope that there will be future opportunities to partner with advocates to truly fulfill the promise of proteomics and "team science." We also recommend that advocates representing the patient perspective be considered an integral part of the "team" to participate as a partner in prioritizing discussions and problem solving, to review educational materials, and to serve as a communications channel to disseminate information about the promise and realities of proteomics to our constituencies. ■

In Vitro Diagnostic Tests for Cancer: Navigating the FDA Approval Process

Jeffrey N. Gibbs, J.D.
Director
Hyman, Phelps & McNamara, PC
Washington, D.C.

The rapid growth in proteomics has provided new insights not only into biological processes but also into tools for creating novel *in vitro* diagnostic (IVD) assays. Researchers are already using proteomic-based assays to generate diagnostic information, and as the knowledge base and technologies advance, the rate of development of new IVDs will increase dramatically.

However, creating and clinically validating proteomic-based IVDs does not necessarily mean that they will be incorporated into medical practice. Discovering, developing, and validating a proteomic-based assay is necessary, but not sufficient, for an assay to be commercialized. There are still other hurdles, including compliance with regulatory requirements.

Under federal law, new IVDs must be reviewed by the Food and Drug Administration (FDA) before they can be sold in the United States. The FDA is the gatekeeper for all new diagnostic assays that are commercially distributed in the U.S. While the FDA has exempted some low-risk, well-understood assays from the need for prior review, proteomic-based assays will not qualify for this exemption.

> *"Discovering, developing, and validating a proteomic-based assay is necessary, but not sufficient, for an assay to be commercialized."*

Developers of proteomic-based diagnostic test kits should expect that they will need to secure FDA marketing authorization before they can launch their kits in the U.S.

There is an alternative route to introducing the assay: offering it as a laboratory developed test. This pathway will be discovered in a separate article in the next issue of *eProtein*.

To complete the FDA review process, companies will need to negotiate multiple steps. Some of the key steps are summarized briefly below.

1. **Identify a specific intended use**: It is difficult to overstate the importance of developing a precisely worded intended use statement at an early stage. The regulatory process is heavily influenced by the intended use. For example, the FDA does not let companies sell a test labeled as "an X Cancer Assay." Rather, the intended use must be more specific and contain information about diagnosis, prognosis, production, screening, monitoring, etc. of a particular type of cancer. The intended use statement may also need to describe how the assay fits into the diagnostic paradigm (e.g., for use by primary care physicians as compared to oncologists). These seemingly subtle word choices can have a major impact on the regulatory process. Even if the wording changes later on, companies need to develop a working intended use statement at the outset.
2. **Developing a protocol**: Companies developing novel proteomic-based IVDs should expect that they will need to conduct a clinical study. The study must be consistent with the proposed intended use. The protocol should carefully address, among other issues, source of specimens, clinical comparator (other FDA-cleared method, clinical diagnosis, etc.), and statistical methodology.
3. **Meeting with the FDA**: In general, there is no obligation to get permission from the FDA before beginning a diagnostic study. Nevertheless, obtaining the FDA's feedback can be extremely helpful, particularly for novel assays. Companies with new kinds of proteomic assays would generally be well served by meeting with the FDA before beginning a clinical validation study. Topics at the meeting could include proposed intended use, key elements of the draft protocol, the regulatory pathway, and statistics. Companies must prepare carefully for these "pre-IDE" (Investigational Device Exemption) meetings and listen carefully to FDA's comments.
4. **Conducting the study**: Because the clinical validation study is intended to support an FDA marketing application, it must meet FDA regulatory requirements, which may entail monitoring study sites and laboratories. Clinical trial agreements are essential; institutional review board (IRB) approval and informed consent may be necessary. The reliability and integrity of the data need to be established.
5. **Conducting preclinical studies**: Companies will also need to conduct a variety of preclinical studies to assess analytical performance. The FDA has developed guidelines relating to preclinical studies. While these are not legally binding, applicants should review the pertinent guidelines before beginning these studies.
6. **Submitting the FDA application**: There are two major routes for obtaining

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Researcher Spotlight:

Next Generation Affinity Reagents for Cancer Biomarker Detection

John C. Chaput, Ph.D.
Research Investigator
The Biodesign Institute at
Arizona State University

> *"Overcoming this problem will likely require transformative ideas that shift the current paradigm..."*

The discovery that tumors leak proteins and peptides into bodily fluids has led to the idea that it may be possible to diagnose cancer at pre-symptomatic stages or access a patient's response to treatment by monitoring specific cancer biomarkers present in human blood and urine. While over 1,000 cancer biomarkers have been described in the literature, only a small fraction of these targets have been independently validated, and an even smaller fraction have a medical diagnostic available for their detection.

One reason for the limited number of validated cancer biomarkers is the lack of high quality affinity reagents needed to detect and bind these targets in complex biological mixtures. Many researchers consider this bottleneck to be a grand challenge in basic and applied biomedicine as the need for high quality affinity reagents is now impacting many large-scale projects that attempt to explore the nature and function of the human proteome. Overcoming this problem will likely require transformative ideas that shift the current paradigm away from methodologies that are costly and time consuming and focus instead on novel solutions that are capable of changing the way in which protein affinity reagents are created.

One approach to relieving the antibody bottleneck is to develop a chemical strategy for making protein affinity reagents that no longer relies on animal immunization or iterative rounds of *in vitro* selection and amplification as the primary means of discovery.

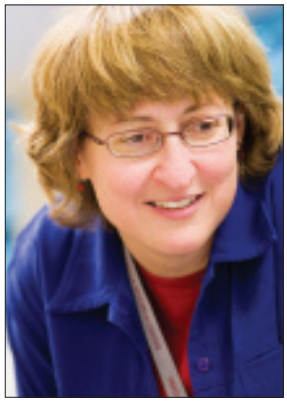
To address this problem, our lab is working to develop a versatile two-step strategy for creating artificial protein affinity reagents that we call *synbodies*. In the first step, non-competing peptide ligands are discovered by array-based or single-pass, high-throughput screening methods that bind to different sites on the surface of a desired protein target. In the second step, combinations of orthogonal peptides are screened on a synthetic DNA scaffold to identify the optimal peptide pair and peptide pair distance required to transform two modest affinity ligands into a single high affinity protein binding agent. This strategy, which is general and amenable to high-throughput, has the potential to become an enabling technology by providing a simple method for creating high quality synthetic antibodies to any water-soluble protein.

In a proof-of-principle demonstration, we generated synbodies to the yeast protein Gal80 and the human blood protein transferrin. In both cases, assembly of the peptides at optimal distances on the DNA scaffold resulted in a synthetic antibody with affinity and specificity similar to a typical antibody. We found that synbodies function in a standard enzyme-linked immunosorbent assay (ELISA) and in an immunoprecipitation assay, which suggests that simple chemical reagents represent a viable alternative to traditional antibodies.

In partnership with CPTC's Advanced Proteomics Platforms and Computational Sciences program, we are applying this technology to develop synthetic affinity reagents to important cancer biomarkers, such as growth factor receptor-bound protein 2 (Grb2), prostate-specific antigen (PSA), and cancer antigen 125 (CA-125). We are particularly interested in evaluating the potential use of synbodies relative to well-characterized monoclonal and polyclonal antibodies. The outcome of these experiments will help establish the general utility of synbodies as future protein affinity reagents for proteomics and cancer research. ■

Researcher Spotlight:

Targeted Proteomics: Relieving a Bottleneck in the Biomarker Pipeline



Amanda Paulovich, M.D., Ph.D.
Assistant Member
Fred Hutchinson Cancer Research Center

Discovering protein biomarker candidates is relatively easy. In fact, hundreds to a thousand protein biomarker candidates are typically discovered at one time using genomic or proteomic technologies such as mass spectrometry. The problem, however, is that most of these candidates are not clinically useful biomarkers. The true biomarkers must be culled from this lengthy list of candidates, which is a very time-consuming, costly, and inaccurate process.

This gap in the biomarker development pipeline—between discovery and clinical validation—results from the lack of available assays for testing candidate biomarkers. This limitation has proven to be a significant barrier for clinical proteomics, and it partly explains why most biomarker candidates never reach clinical testing. A faster and less expensive assay is needed in order to reduce the time and cost of evaluating novel potential cancer diagnostic biomarkers.

Amanda Paulovich, M.D., Ph.D., Assistant Member of the Fred Hutchinson Cancer Research Center and a member of the Clinical Proteomic Technology Assessment for Cancer (CPTAC) Network, is developing a verification technology that will help to determine which biomarker candidates are worth pursuing in the clinic. Specifically, her laboratory is collaborating closely with Drs. Leigh Anderson (Plasma Proteome Institute), Steve Carr (The Broad Institute of MIT and Harvard), Terry Pearson (University of Victoria), and Steve Skates (Massachusetts General Hospital) to test a targeted type of mass spectrometry (MS), called multiple reaction monitoring (MRM-MS), that can measure specific proteins in a highly multiplexed fashion. Combined with an enrichment technology, stable isotope standards and capture by anti-peptide antibodies (SISCAPA), MRM assays achieve sufficient sensitivity for measuring candidate protein biomarkers in the ng/ml or lower range in plasma.

"These assays are faster and cheaper to develop than conventional immunoassays, and the mulitplex capability [ability to test multiple biomarkers in one test] is quite high," explains Paulovich. "We are able to test far larger numbers of candidates than has been possible in the past, presumably improving our odds of identifying clinically useful markers."

SISCAPA-MRM-MS may serve as the much needed bridge between biomarker discovery and clinical validation. Restructuring the biomarker development pipeline with the addition of this new assay technology will ensure that only the strongest biomarker candidates will move into clinical validation. "This assay technology is still at an early stage and is undergoing refinement to make it more acceptable for potential clinical applications, which is what our group is really focused on," says Paulovich.

> *"This assay technology is still at an early stage and is undergoing tremendous refinement to make it more acceptable for potential clinical applications, which is what our group is really focused on."*

Paulovich, along with Jeff Whiteaker, Ph.D., and Lei Zhao in her laboratory, recently participated in a collaborative effort within the CPTAC network, demonstrating the reproducibility of MRM-based assays across laboratories, which is a critical characteristic for detection of disease-specific biomarkers. This study was published in the July 2009 issue of *Nature Biotechnology*.

A Multi-site Assessment of Precision and Reproducibility of Multiple Reaction Monitoring-based Measurements By the NCI-CPTAC Network: Toward Quantitative Protein Biomarker Verification in Human Plasma. Nat. Biotechnol. [Epub 2009 Jun 28]

Industry News

Study Supports New Bridge Technology for Biomarker Development Pipeline: MRM-MS

A team of CPTAC researchers has demonstrated that a new method for detecting and quantifying protein biomarkers in body fluids, multiple reaction monitoring mass spectrometry (MRM-MS), may ultimately make it possible to screen multiple biomarkers in hundreds of patient samples, thus ensuring that only the strongest biomarker candidates will advance down the development pipeline. The goal of this research is to reduce the time and cost of developing cancer diagnostic tests, ultimately increasing the number of such tests in the clinic so cancer can be caught at its earliest stages.

"These findings are significant because they provide a potential solution for eliminating one of the major hurdles in validating protein biomarkers for clinical use. Thousands of cancer biomarkers are discovered every day, but only a handful ever makes it through clinical validation. This is a critical roadblock because biomarkers have the potential to allow doctors to detect cancer in the earliest stages, when treatment provides the greatest chances of survival," says John E. Niederhuber, M.D., NCI director. "The critical limiting factor to date in validating biomarkers for clinical use has been the lack of standardized technologies and methodologies in the biomarker discovery and validation process, and this research may solve that dilemma."

The study results were published in the online version of *Nature Biotechnology* on June 28, 2009. Click here to read the full press release. Click here to read coverage in the NCI Cancer Bulletin.

imaGenes to Distribute Highly Characterized Monoclonal Antibodies Produced by CPTC

imaGenes (www.imagenes-bio.de), a premier provider of genome research services in Europe, will distribute monoclonal antibodies created and characterized by CPTC. CPTC's antibody characterization program, a component of its Proteomic Reagents and Resources core, uses standard operating procedures to create highly characterized monoclonal antibodies to human proteins associated with cancer for research use (http://antibodies.cancer.gov). "This resource will accelerate biomarker discovery and validation and will ultimately assist to rapidly advance the use of new biomarkers in clinical practice," says Johannes Maurer, imaGenes' Director of Genomic Products & Marketing.

Advancing Principles for Data Sharing by Proteomics Researchers

Leaders in proteomics are pushing to develop a set of principles to guide data sharing in this field. A *Journal of Proteome Research paper*, which resulted from the 2008 International Summit on Proteomics Data Release and Sharing Policy, held in Amsterdam, outlines the challenges facing such efforts.

Read the full story in the *Journal of Proteome Research*. *No subscription is required*.

In Vitro Diagnostic Tests for Cancer: Navigating the FDA Approval Process *(continued from page 4)*

FDA marketing authorization. The 510(k) premarket notification requires the applicant to demonstrate that its assay is "substantially equivalent" to a legally marketed "predicate device," i.e., a device cleared by the FDA or marketed before May 28, 1976. (The latter option is unlikely here.) The key is finding another 510(k)-cleared device with the same or roughly similar intended use. The other primary route is the premarket approval application (PMA). PMAs are more complicated than 510(k)s and also are subject to more controls and regulatory requirements once on the market. In general, the 510(k) route will be preferred. Both kinds of applications require careful preparation and attention to detail. A third alternative is the "*de novo* automatic classification," but that has been used in only a very small percentage of applications.

Once the FDA receives a 510(k), they can approve it (technically called a "clearance"), ask questions, or reject it (found "not substantially equivalent"). The FDA will respond to the 510(k) within ninety days of submission. If they ask for more information, the ninety-day clock may reset upon receipt of the company's reply. For PMAs, the outcomes are similar: approval, ask questions, or disapproval. The review clock for PMAs is 180 days.

Getting clearance or approval does not discharge all regulatory obligations. Once an IVD is on the market, companies must comply with multiple FDA post-marketing regulatory requirements.

Proteomic-based technologies offer exciting opportunities for improving clinical diagnoses. However, before these tests can be offered commercially as kits, they will need to successfully navigate the FDA review process. Just as the development of the assay itself requires careful planning and scientific rigor, so does the FDA process. ■

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Advancing Protein Science for Personalized Medicine

Upcoming Events

September 26-30, 2009
HUPO VIII World Congress
The Westin Harbour Castle
Toronto, Canada

October 5-7, 2009
Clinical Proteomic Technologies for Cancer Annual Meeting: Advancing Protein Science for Personalized medicine
Hyatt Regency Bethesda
Bethesda, Md

October 7-9, 2009
Innovative Molecular Analysis Technologies (IMAT) Program Meeting
Organized by: NCI
Hyatt Regency Bethesda
Bethesda, Md

November 5-6, 2009
Translating Novel Biomarkers to Clinical Practice: Role and Opportunities for the Clinical Laboratory
Hosted by: American Association for Clinical Chemistry (AACC)
The Marriott Bethesda Hotel & Conference Center
Bethesda, Md

For a full list of upcoming events, visit http://proteomics.cancer.gov/mediacenter/events.

Contact Information

For more information about the CTPC, please visit http://proteomics.cancer.gov, or contact us at:

National Cancer Institute
Office of Technology & Industrial Relations
ATTN: Clinical Proteomic Technologies for Cancer
31 Center Drive, MSC 2580
Bethesda, Md 20892-2580
Email: cancer.proteomics@mail.nih.gov

The NCI Clinical Proteomic Technologies for Cancer initiative seeks to foster the building of an integrated foundation of proteomic technologies, data, reagents and reference materials, and analysis systems to systematically advance the application of protein science to accelerate discovery and clinical research in cancer.

Reagents Data Portal

http://antibodies.cancer.gov
http://dshb.biology.uiowa.edu

Newly Released Antigens and Antibodies

Antigen	Antibody
14-3-3 Sigma	CPTC-SFN-1 CPTC-SFN-2 CPTC-SFN-3
BCL2-like 2	CPTC-BCL2L2-1 CPTC-BCL2L2-2 CPTC-BCL2L2-3
Calcyclin	CPTC-Calcyclin-1 CPTC-Calcyclin-2
Chloride Intracellular Channel 1	CPTC-CLIC1-1 CPTC-CLIC1-2
Fascin	CPTC-Fascin-1 CPTC-Fascin-2 CPTC-Fascin-3
Glutathione S Transferase M1	CPTC-GST M1-5 CPTC-GST M1-6 CPTC-GST M1-7
Melanoma Antigen Family A, 4	CPTC-MAGEA4-1 CPTC-MAGEA4-2 CPTC-MAGEA4-3
MethylCpG Binding Protein 1	CPTC-MBD1-1 CPTC-MBD1-2 CPTC-MBD1-3
Protein Phosphatase 2A	CPTC-PP2A-1 CPTC-PP2A-2 CPTC-PP2A-3 CPTC-PP2A-4
Ubiquitin conjugating enzyme E2C	CPTC-UBE2C-1